21-[^{18}F]Fluoro-16α-ethyl-19-norprogesterone: Synthesis and Target Tissue Selective Uptake of a Progestin Receptor Based Radiotracer for Positron Emission Tomography

Martin G. Pomper,† John A. Katzenellenbogen,*† Michael J. Welch,‡ James W. Brodack,‡ and Carla J. Mathias‡

Department of Chemistry, University of Illinois, Urbana, Illinois 61801, and Division of Radiation Sciences, Mallinckrodt Institute of Radiology, Washington University Medical School, St. Louis, Missouri 63110. *Received November 9, 1987*

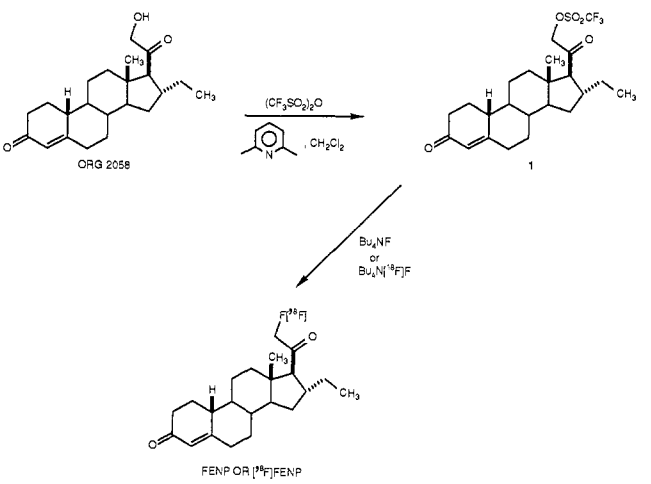
We have synthesized 21-[^{18}F]fluoro-16α-ethyl-19-norprogesterone (FENP), a high affinity ligand for the progesterone receptor, labeled with the positron-emitting radionuclide fluorine-18 ($t_{1/2}$ = 110 min). The synthesis proceeds in two steps from 21-hydroxy-16α-ethyl-19-norprogesterone and involves [^{18}F]fluoride ion displacement of the 21-trifluoromethanesulfonate (21-triflate). This material is purified by HPLC and is obtained in 4–30% overall yield (decay corrected) within 40 min after the end of bombardment to produce [^{18}F]fluoride ion. The effective specific activity, determined by competitive radioreceptor binding assays, is 700–1400 Ci/mmol. In vivo, [^{18}F]FENP demonstrates highly selective, receptor-mediated uptake by the uterus of estrogen-primed rats; the uterus to blood and uterus to muscle ratios were respectively 26 and 16 at 1 h and 71 and 41 at 3 h after injection. The high target tissue selectivity of this uptake suggests that this compound may be useful for the in vivo imaging of progestin target tissues and receptor-rich tumors (such as human breast tumors) by positron emission tomography.

Steroid hormone receptors, present in target tissues and receptor-positive tumors, form the basis for the selective uptake of suitable steroid radiopharmaceuticals that could be used for in vivo imaging by emission tomography. Applications of particular interest are the imaging of receptor-positive breast[1] and ovarian tumors[2] and target tissues in the brain.[3]

Recently, we have described the preparation of 16α-[^{18}F]fluoroestradiol and its selective uptake by estrogen target tissues in rats and its use in positron emission tomography of breast tumors in humans.[4] In principle, a progestin-based imaging agent for breast tumors might be preferred over an estrogen-based agent because response to hormonal therapy is more significantly correlated with progesterone receptor positivity than with estrogen receptor positivity.[5] Furthermore, estrogen receptor positive tumors in patients on hormonal therapy (e.g., tamoxifen) could not be imaged with an estrogen, since the circulating levels of tamoxifen and its metabolites are sufficiently high to fully occupy the estrogen receptor.[6] In contrast, progestin receptors in tumors of patients on tamoxifen should be unoccupied, and their levels might even be increased by the hormone therapy.[7]

Earlier investigations on positron-emitting progestins generally resulted in the preparation of compounds with low affinity for the receptor or low specific activity; hence, little target tissue selectivity was observed in vivo.[8] As a result of our recent studies on receptor binding and in vivo uptake of a fluoromethyl derivative of norethisterone[9] and three tritium-labeled progestins (progesterone, R 5020, and ORG 2058),[10] we concluded that target to nontarget tissue activity ratios suitable for imaging would only be achieved with ligands having receptor affinities significantly greater than that of progesterone itself.[1] 21-Fluoro-16α-ethyl-19-norprogesterone (FENP),[11] an analogue of the potent progestin ORG 2058[11] in which the 21-hydroxy group is replaced by a fluorine atom, is also known to be a high-affinity, potent progestin (Scheme I).[12] In this paper we describe a simple, two-step preparation of FENP from ORG 2058 that we have adapted to fluorine-18 labeling. [^{18}F]FENP prepared by this route shows highly selective uptake by the uterus of estrogen-primed immature rats and thus appears to be an excellent candidate for imaging progesterone receptor positive tumors of the breast.

Scheme I. Preparation of FENP and [^{18}F]FENP



* Address correspondence to: John A. Katzenellenbogen, 461 Roger Adams Laboratory, Box 37, 1209 W. California St., Department of Chemistry, University of Illinois, Urbana, IL 61801.
† University of Illinois.
‡ Washington University Medical School.

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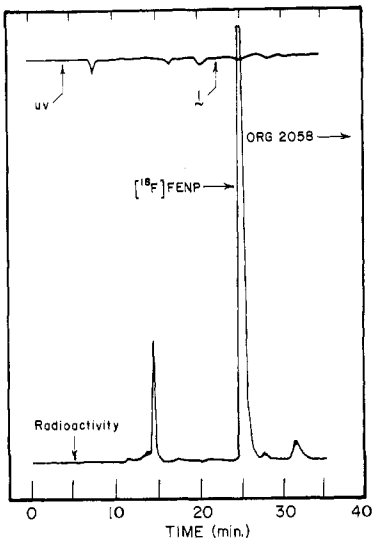

Figure 1. HPLC trace of purification of [^{18}F]FENP. Analysis was performed on preparative 10-μm silica gel HPLC column (Whatman Partisil M9, 0.9 × 50 cm) eluted with hexane/CH_2Cl_2/*i*-PrOH (70:28.5:1.5) at 3 mL/min. UV detection was at 254 nm (0.1 absorbance units full scale); radioactivity was detected with flow-through scintillation detector. The large radioactivity peak at ~25 min is [^{18}F]FENP. Compound 1 normally elutes at ~22 min. ORG 2058 only elutes when the column is washed with a more polar solvent (hexane/CH_2Cl_2/*i*-PrOH, 10:85.5:4.5). This reaction employed 1.1 mCi of $^{18}F^-$.

Results and Discussion

Synthesis of FENP by Fluoride Ion Displacement. The preparation of FENP from ORG 2058 is illustrated in Scheme I. ORG 2058 is converted to the 21-trifluoromethanesulfonate (triflate) derivative 1 by treatment with triflic anhydride and 2,6-lutidine in dichloromethane at −78 °C for 1 h. After removal of the solvent, this material is purified by flash chromatography[13] (30% ethyl acetate in hexane) and is obtained as a colorless solid. Although triflate esters are highly reactive, triflate 1 can be stored at −25 °C under N_2 for indefinite periods without significant decomposition.

The preparation of FENP from triflate 1 involves treatment with *n*-Bu_4NF in THF at 60 °C for 10 min. Yields of FENP with excess fluoride ion are in the range of 50–60% (estimated by HPLC analysis; cf. Figure 1 for conditions; calibration achieved with progesterone as an internal standard). Useful yields (ca. 30%) are still obtained when fluoride ion is the limiting reagent (0.1 equiv); however, excess triflate then elutes just ahead of FENP by HPLC (retention times, FENP = 25 min; 1 = 22 min) and might, therefore, compromise the effective specific activity of the final [^{18}F]FENP.[14] We have found, however, that this problem can be avoided by the addition of a 2.5-fold excess of *n*-Bu_4NOH to the reaction mixture prior to product isolation; this hydrolyzes excess triflate to ORG 2058 quantitatively within 2 min at 20 °C and results in a purer product.[15]

Table I. Tissue Distribution of ^{18}F Activity after Injections of [^{18}F]FENP into Estrogen-Primed Immature Rats[a]

	percent injected dose[b]/g ± SD,[c] n = 5			
tissue	1 h	1 h (low dose)	1 h (blocked)	3 h
uterus	6.43 ± 1.53	4.03 ± 0.37	0.38 ± 0.04	4.63 ± 1.24
ovaries	2.86 ± 0.78	2.37 ± 0.29	1.00 ± 0.36	1.72 ± 0.73
blood	0.25 ± 0.07	0.19 ± 0.03	0.15 ± 0.02	0.07 ± 0.02
muscle	0.42 ± 0.10	0.39 ± 0.13	0.25 ± 0.08	0.12 ± 0.04
lung	0.47 ± 0.20	0.57 ± 0.8	0.30 ± 0.02	0.12 ± 0.01
brain	0.63 ± 0.16	0.48 ± 0.05	0.32 ± 0.02	0.14 ± 0.05
liver	3.34 ± 0.90	2.59 ± 0.73	2.09 ± 0.29	1.01 ± 0.25
kidney	1.05 ± 0.22	0.86 ± 0.11	0.58 ± 0.04	0.33 ± 0.09
fat	3.56 ± 0.66	4.13 ± 0.65	2.90 ± 0.79	2.78 ± 0.57
bone	3.18 ± 0.76	3.06 ± 0.94	3.09 ± 0.41	5.55 ± 0.46
uterus/blood	26.26 ± 6.58	21.18 ± 3.76	2.53 ± 0.34	71.15 ± 21.4
uterus/muscle	15.98 ± 5.51	11.06 ± 2.92	1.59 ± 0.47	41.16 ± 13.1

[a] Female Sprague–Dawley rats (21 days old) were estrogen primed by injection ip with 5 μg of estradiol in 0.1 mL of 5% ethanol–sunflower oil on three successive days and used on the fourth day (24 days old), 24 h after the last injection. Average animal weight at time of experiment was 73 ± 6 g. [b] Normal dose: 120 μCi of [^{18}F]FENP in 0.1 mL of 10% ethanol–saline; "low dose" 13 μCi; for "blocked" animals, ≅17 μg of FENP were added to each dose of [^{18}F]FENP, injection volume was 0.2 mL. [c] SD is standard deviation.

Progesterone Receptor Binding Affinity of FENP. The binding affinity of FENP to the progesterone receptor was measured by a competitive radiometric binding assay with estrogen-primed immature rat uterus as a source of progesterone receptor, [^{3}H]R 5020 as tracer, and dextran-treated charcoal as adsorbant for free ligand.[16] The following binding affinities were found (relative to progesterone = 100%): R 5020 = 770%, ORG 2058 = 1500%, FENP = 6000%. The equilibrium dissociation constant (K_d) for progesterone in this system is 3 nM.

Synthesis of [^{18}F]FENP. For the preparation of [^{18}F]FENP, *n*-$Bu_4N^{18}F$ was prepared by proton bombardment of an oxygen-18 enriched water target (^{18}O(p,n)^{18}F)[17] followed by the addition of 2–3 μmol of *n*-Bu_4NOH and careful evaporation of water and then azeotropic drying with acetonitrile, as previously described.[18] The *n*-Bu_4N[^{18}F]F could be resolubilized in acetonitrile in >80% yield. Triflate 1 was added, and the reaction temperature was maintained at 60 °C for 8 min; the mixture was cooled to 20 °C, and *n*-Bu_4NOH was added.[15] The product was then isolated and purified by HPLC.

A trace, showing the profile of radioactivity and UV absorbance, is given in Figure 1. The elution positions of authentic FENP and triflate 1, as well as ORG 2058, are indicated. It is clear that [^{18}F]FENP is obtained with little, if any, coeluting UV absorbing impurities evident. The overall radiochemical yield after HPLC purification is 4–30% (decay corrected at 40 min from EOB); reaction scale ranged from 1 to 160 mCi. By HPLC, the estimated specific activity is at least 100 Ci/mmol; a more selective measurement of the effective specific activity by competitive binding assays on a decayed sample of known radioactivity[14] gave a value of ca. 700–1400 Ci/mmol.

(11) The following abbreviations are used: FENP (also known as ORG OH-06) for 21-fluoro-16α-ethyl-19-norprogesterone (IUPAC name: 21-fluoro-16α-ethyl-19-norpregn-4-ene-3,20-dione); ORG 2058 for 21-hydroxy-16α-ethyl-19-norpregn-4-ene-3,20-dione; R 2050 (promegestrone) for 17α,21-dimethylpregna-4,9(10)-diene-3,20-dione.

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(15) In the preparation of FENP or [^{18}F]FENP, the overall yield is reduced by 30–60% by this quenching procedure, but in the preparation of [^{18}F]FENP, the effective specific activity is increased.

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In Vivo Uptake of [^{18}F]FENP in Estrogen-Primed Immature Rats. Purified [^{18}F]FENP was reconstituted in 10% ethanol–saline, and 130-μCi portions were injected (iv, femoral vein) into estrogen-primed 24-day-old female Sprague–Dawley rats. Tissue distribution of fluorine-18 radioactivity was determined at 1 and 3 h (see Table I). To ascertain whether the uptake was mediated by a high-affinity, limited-capacity system, one set of animals was given ~17 μg of unlabeled FENP together with [^{18}F]FENP; this dose of FENP is sufficient to fully occupy the progesterone receptors and thus should block the uptake of [^{18}F]FENP (Table I, 1 h "blocked"). To establish that target tissue uptake of [^{18}F]FENP was not being limited by undetected progestin receptor binding impurities, one set of animals was also injected with only 13 μCi of [^{18}F]FENP (Table I, 1 h "low dose").

From these data (Table I), it is clear that the uterine uptake of [^{18}F]FENP is high and selective; uterus to blood and uterus to muscle (nontarget tissue) ratios dramatically increase between 1 h and 3 h. Comparable uptake is observed at 1 h with both the normal and the low doses, indicating that target tissue uptake is not limited by sample specific activity. Receptor-mediated uptake in the uterus is indicated by the decreased uterine uptake in the blocked animals. The activity in the uterus remains quite high even at 3 h, consistent with the high receptor content of this tissue (60 nM) and the high progestin receptor binding affinity of FENP (6000% relative to progesterone); selective uptake by the ovaries is also observed. Relative to muscle, lung, and blood, there is considerable activity in the liver and kidney. This is consistent with the known organ sites of metabolism and routes of excretion of steroids in rodents.[19]

There is also considerable uptake into fat and bone. Fat uptake is observed with lipophilic compounds and is considerably greater with [^{18}F]FENP than previously observed with the less lipophilic ^{18}F-labeled estrogens.[4,20] In our recent study,[10] considerable fat uptake was also observed with [^{3}H]R 5020 and [^{3}H]progesterone, but less with the more hydrophilic [^{3}H]ORG 2058. Since adipose tissue constitutes a significant fraction of the breast, this uptake into fat may reduce the selectivity of receptor-mediated accumulation of [^{18}F]FENP by a progestin receptor positive breast tumor vs normal breast tissue. This point will require further evaluation in primates.

Uptake in bone indicates that [^{18}F]fluoride ion is being generated metabolically, as might be expected by reasonable metabolic cleavages. Since bone is a relatively radioinsensitive organ and fluorine-18 has a low effective dose equivalency and a short half-life ($t_{1/2}$ = 110 min), the bone uptake should not provide radiation dosimetry problems.[21]

Conclusion

21-Fluoro-16α-ethyl-19-norprogesterone (FENP) is a high-affinity ligand for the progesterone receptor that can be readily prepared labeled with fluorine-18 at high specific activity. In estrogen-primed immature rats, [^{18}F]FENP shows highly selective uptake for the target tissue, suggesting that this compound might be useful for the in vivo imaging of target sites for progestins, including progesterone receptor positive tumors of the breast, by positron emission tomography.

Experimental Section

General Methods. Melting points were determined on an Electrothermal apparatus and are uncorrected. Flash chromatography was performed according to Still,[13] with Woelm 32–63-μm SiO_2. Solvents and column packing dimensions are indicated parenthetically. Proton nuclear magnetic resonance (^{1}H NMR) spectra were recorded on a Varian XL-200 (200 MHz) spectrometer; chemical shifts are reported in ppm downfield from a tetramethylsilane internal reference. The ^{1}H NMR data are reported in the form: δ value of signal (peak multiplicity, number of protons, coupling constant (if applicable)). A V. G. Instruments ZAB HF mass spectrometer provided spectra via fast atom bombardment (FAB) employing a dithiothreitol matrix; data are reported in the form: m/z (intensity relative to base peak = 100). High-performance liquid chromatography (HPLC) was performed on a Spectra-Physics Model 8700 liquid chromatograph with a 5-μm analytical SiO_2 column (4.6 mm × 30 cm, Varian Si-5 Micro Pak) or a 10-μm preparative SiO_2 column (Whatman Partisil M-9, 0.9 cm × 50 cm). The eluant was monitored with a variable wavelength detector set at 254 nm (0.1 absorbance unit full scale) and a flow-through sodium iodide scintillation detector, where appropriate. Radioactivity was determined with a dose calibrator.

21-[[(Trifluoromethyl)sulfonyl]oxy]-16α-ethyl-19-norpregn-4-ene-3,20-dione (1). In a nitrogen-filled glove bag, a glass vial was charged with ORG 2058 (Organon Corp., Oss, The Netherlands) (50 mg, 0.15 mmol). After dissolution in freshly distilled (CaH_2) CH_2Cl_2 (1.25 mL), the mixture was placed in a dry ice/EtOH bath (−78 °C). Trifluoromethanesulfonic anhydride (37 μL, 0.22 mmol) followed by 2,6-lutidine (26 μL, 0.22 mmol) was added. The deep orange solution was allowed to stir for 1 h, at which time cold THF (1.25 mL) was added followed by the subsequent addition of 2 mL each of cold distilled water and cold CH_2Cl_2. The aqueous and organic phases were separated, and the organic layer was dried over Na_2SO_4. Passage through neutral alumina and subsequent concentration in vacuo gave a bright orange oil, which was purified by flash chromatography (30 mm × 15 cm SiO_2, 30% EtOAc/hexane). This afforded 53.5 mg (78%) of an off-white, air-sensitive solid: mp 68–72 °C dec; ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.78 (s, 3 H, 18-CH_3), 0.82 (t, 3 H, J = 7.8 Hz, 16α-CH_2CH_3), 4.89 (d, 2 H, J = 3.6 Hz, 21-H), 5.84 (br s, 1 H, 4-H); FABMS, 477 (M^+ + H, 100), 329 (20), 195 (31), 159 (19), 135 (25), 119 (70). Anal. (exact mass, HR-FABMS). Calcd for $C_{23}H_{31}SO_5F_3$ m/z 476.1902, found 476.1902.

21-Fluoro-16α-ethyl-19-norpregn-4-ene-3,20-dione (FENP). Triflate 1 (1.05 mg, 2.20 μmol) was dissolved in 200 μL of freshly distilled THF (sodium benzophenone ketyl) in a glass vial. n-Bu_4NF (0.22 μmol) (2.2 μL of a 0.10 M solution (Aldrich)) was added, and the reaction mixture was allowed to stir for 10 min at 60 °C. Progesterone (0.078 μmol) was added as an internal standard. Solvent was removed in vacuo, the residue was dissolved in 50 μL of CH_2Cl_2, and 3 μL of the resulting solution was injected onto a preparative HPLC column (70% hexane, 28.5% CH_2Cl_2, 1.5% i-PrOH, 3 mL/min). A 43% yield of FENP was obtained, relative to internal standard (progesterone). Unreacted 1 eluted first (t_R 16.2 min) followed by FENP (t_R 17.5 min) and then progesterone (t_R 20.1 min). FENP produced in this reaction was shown to coelute with authentic FENP (Organon) in this system as well as by reversed-phase preparative HPLC (80% CH_3CN, 20% H_2O, 3 mL/min): FABMS, 347 (M^+ + H, 60), 242 (100), 184 (21), 152 (24), 142 (31), 199 (44). Anal. (exact mass, HR-FABMS). Calcd for $C_{21}H_{31}O_2F$ m/z 347.2379, found 347.2379.

21-[^{18}F]Fluoro-16α-ethyl-19-norpregn-4-ene-3,20-dione ([^{18}F]FENP). Fluorine-18 was prepared from [^{18}O]H_2O as previously described.[17] The aqueous activity was added to 3.7 μmol of n-Bu_4NOH and taken to dryness under a stream of nitrogen at 110 °C in a Vacutainer. The residue was further dried by two azeotropic distillations with 100-μL portions of CH_3CN. Approximately 80% of the dried activity (84 mCi) was resolubilized in 200 μL of CH_3CN and transferred to a glass vial containing approximately 1.5 mg (3.0 μmol) of 1. After 8 min at 60 °C, the reaction mixture was passed through a 0.5-cm plug of neutral alumina. At this point, 8 μmol of n-Bu_4NOH was added,

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solvent was removed under a stream of nitrogen, and the residue was dissolved in 1 mL of CH_2Cl_2 and injected onto a preparative HPLC column, as for FENP, to yield 2.5 mCi (4.3%, decay corrected) of [^{18}F]FENP (t_R 25 min). See Figure 1 for a radio- and UV chromatogram for a similar, though smaller scale, reaction.

Acknowledgment. We are grateful for support of this work through grants from the Department of Energy (Grant DE-FG02-86ER60401 to J.A.K. and Grant DE-FG02-84ER60218 to M.J.W.) and the National Institutes of Health (Grant HL13851). We are thankful to Dr. F. Zeelen, of Organon, for providing us with samples of ORG 2058 and ORG OH-06 (FENP) and to K. E. Carlson for assistance.

Registry No. 1, 113975-10-3; ORG 2058, 24320-06-7; FENP, 25908-76-3; [^{18}F]FENP, 113948-68-8.

Cardiotonic Agents. Synthesis and Inotropic Activity of a Series of Isoquinolin-3-ol Derivatives

Ramesh M. Kanojia, Jeffery B. Press,* O. William Lever, Jr.,* Louella Williams, James J. McNally, Alfonso J. Tobia, Robert Falotico, and John B. Moore, Jr.

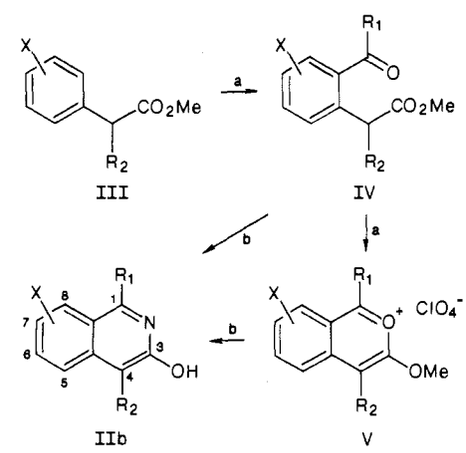
Research Laboratories, Ortho Pharmaceutical Corporation, Raritan, New Jersey 08869. *Received December 7, 1987*

A series of isoquinolin-3-ol derivatives (II) was prepared as analogues of the clinical cardiotonic agent bemarinone (ORF 16600, I). Although in many respects the structural requirements for the cardiotonic activity of II are similar to those of bemarinone, certain differences between the series were noted. Our structure–activity studies show that II is less sensitive to alkoxy-substitution effects than is I, and more significantly, 4-substitution of II by alkyl groups, halogen, or alkanecarboxylic acid derivatives enhances cardiotonic activity in II in contrast to I, wherein analogous substitution eliminated activity. A linear correlation between contractile force (CF) increase and cyclic nucleotide phosphodiesterase fraction III (PDE-III) inhibition by the title compounds was determined. The isoquinoline derivatives were characteristically short-acting cardiotonic agents with good potency and selectivity.

Our laboratory has been investigating novel quinazolinones for a number of years,[1] and recently we reported the synthesis[2] and cardiotonic activity[3,4] of bemarinone (ORF 16600, I). Compound I was the most potent cardiotonic agent of the series in which structure–activity relationship (SAR) studies revealed that (1) 5,6-dimethoxy and 5,6-methylenedioxy substitution produced the best activity, (2) hydrogen at N-1 (rather than alkyl) was essential, (3) there was moderate bulk tolerance at C-4 with methyl, ethyl, and isopropyl substitution, all producing good activity, and (4) a good correlation was found between cardiotonic activity and phosphodiesterase fraction III (PDE-III) inhibition although PDE-III inhibition alone was not predictive of cardiotonic activity for this series.

We became interested in evaluating related compounds that were isosteric with I. In particular, isoquinoline analogues such as II in which the N-1 position is replaced by a carbon were especially interesting since methods of synthesis for such systems are known[5] and several alkaloid isoquinoline derivatives such as papaverine, dioxyline, and ethaverine,[6] as well as some related derivatives,[7] are known to possess interesting cardiovascular pharmacology. Compounds of structure II may exist as a lactam (IIa) or lactim (IIb) tautomer among other possibilities,[8,9] either or all of which might have interesting pharmacological properties. The lactim form appears to be preferred in nonhydroxylic solvents,[9] although this tautomerism also depends upon substitution on the heterocyclic ring. Of particular interest for our study are the various alkoxy-

Scheme I[a]



[a] (a) $(R_1CO)_2O/HClO_4/0$ °C to room temperature, (b) NH_4OH or NH_4OAc.

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